ANNEX

From Brockhoff et al J Agric Food Chem. 2007 Jul 25;55(15):6236-43. Epub 2007 Jun 27.

from Dotson CD et al. PLoS One. 2008;3(12):e3974. Epub 2008 Dec 18.

Plasmids containing *TAS2R9* cDNAs were transiently transfected into HEK293 cells stably expressing the chimeric G protein subunit $G_{16gust44}$ [59] using TransIT-293 (Mirus Corporation), according to the manufacturer's protocol. Cells were plated into 384- well plates and after 24–30 hr loaded for 1 h with the calcium-sensitive dye Fluo4-AM and stimulated with bitter compounds. Calcium signals were recorded simultaneously from each well after excitation at 488 nm. The obtained signals (F) were normalized to the fluorescence of cells before stimulation (Fo) and expressed as $\Delta F/F$ value: $\Delta F/F = (F-Fo) / Fo$. Responses of four wells containing cells expressing the same receptor and receiving the same stimulus were averaged. Calculations were based on at least three independent transfection experiments.

fromm Pronin et al. Curr Biol. 2007 Aug 21;17(16):1403-8.

based on our own and published data [S11, S12]) were tested.

Plasmids containing *hT2R* cDNAs were transiently transfected into HEK293 cells stably expressing the chimeric G protein subunit Gα16 gust44 [S13] with TransIT-293 (Mirus), according to the manufacturer's protocol. Cells were plated into 384-well plates and after 24–30 hr were loaded for 1 hr with the calcium-sensitive dye Fluo-4, AM and stimulated with bitter compounds. We recorded calcium signals simultaneously from each well after excitation at 488 nm. The obtained signals (F) were normalized to the fluorescence of cells before stimulation (Fo) and expressed as a $\Delta F/F$ value: $\Delta F/F = (F - Fo)/Fo$. We averaged responses of four wells containing cells expressing the same receptor and receiving the same stimulus. Our calculations were based on at least three independent transfection experiments. Concentration-response curves and EC_{50} values were calculated in GraphPad Prism by nonlinear regression.

from Sakurai et al. J Agric Food Chem. 2009 Mar 25;57(6):2508-14.

Figure 1. Response of hTAS2R16 to salicin in the presence of peptides, amino acids, or amino acid salts. (A) Representative ratiometric images of fura-2 loaded HEK293T cells coexpressing hTAS2R16 and Gα16gust44 in response to 1 mM salicin in the absence or presence of 10 mM peptides, amino acids, or amino acid salts. The top and bottom columns show the representative cell images obtained before and after ligand application, respectively. The color scale indicates the F340/F380 ratio as a pseudocolor. (B) Responses of the HEK293T cells coexpressing hTAS2R16 and Gα16gust44 to 1 mM salicin in the absence and presence of 10 mM peptides, amino acids, or amino acid salts. Each of the columns represents the percentage of the responsive cells in randomly selected DsRed-positive cells (mean ± SE from at least 3 independent measurements). The significance of the differences between the control (1 mM salicin) and test values were tested by using one-way ANOVA followed by the Dunnett's test. ***, $p < 0.001$ vs salicin.